1626

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Hans Reichenbach *et al.*
Serial No.: 09/313,524
Filed: May 17, 1999
For: EPOTHILONES C, D, E AND F PREPARATION AND COMPOSITIONS

Examiner: Solola
Art Unit: 1626

Request for Amendment of Inventorship Under 37 CFR 1.48(b)

The prosecution of the present nonprovisional application has resulted in the amendment or cancellation of claims so that fewer than all of the currently named inventors are the actual inventors of the invention now being claimed.

Accordingly this amendment is being filed pursuant to 37 CFR §1.48(b) to request deletion of the names of HANS REICHENBACH and KLAUS GERTH who are not inventors of the invention now being claimed. Upon the granting of this request, the inventors of the invention now being claimed are GERHARD HOEFLE and HEINRICH STEINMETZ

The undersigned acknowledges that the Invention of HANS REICHENBACH and KLAUS GERTH is no longer being claimed in this application.

The processing fee set forth in 37 CFR § 1.17(i) is annexed hereto. In the event this fee is deemed inadequate, authority is hereby given to charge any deficiency to Deposit Account No. 13-2165.

Respectfully Submitted,
MATHEWS, COLLINS, SHEPHERD & McKAY, P.A.
Attorneys for Applicants

By:
Christopher Casieri
Reg. No. 50,919

May 20, 2004

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I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: NonFEE AMENDMENT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on June 10, 2004.

6-10-04 (Name) Mickey Cherry (Signature)

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AMENDMENT

Sir:

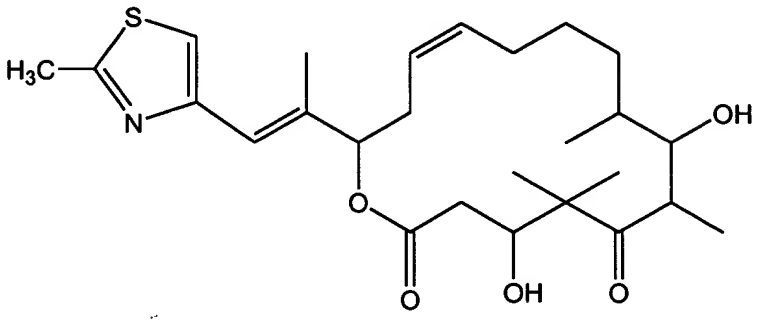
Kindly amend this application as follows:

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application

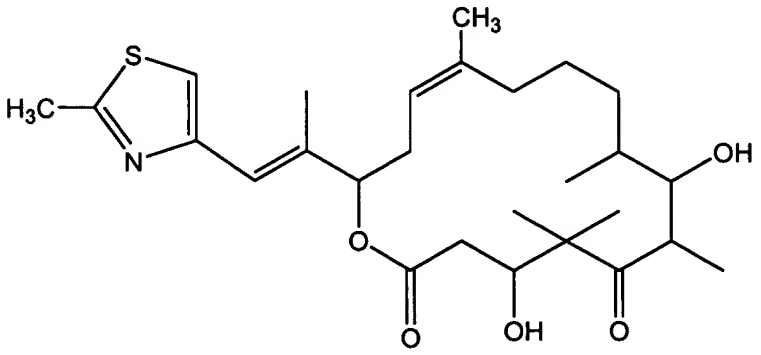
Listing of Claims:

Claim 1. (Amended) A compound according to claim 20 in which R is hydrogen, said compound having the formula:



Claim 2. (Amended) A compound according to claim 1 sufficiently purified to exhibit the 1H- and 13C-NMR spectrum of Table 1 for epothilone C.

Claim 3. (Amended) A compound according to claim 20 in which R is methyl, said compound having the formula:



Claim 4. (Amended) A compound according to claim 3 sufficiently purified to exhibit the 1H- and 13C-NMR spectrum of Table 1 for epothilone D.

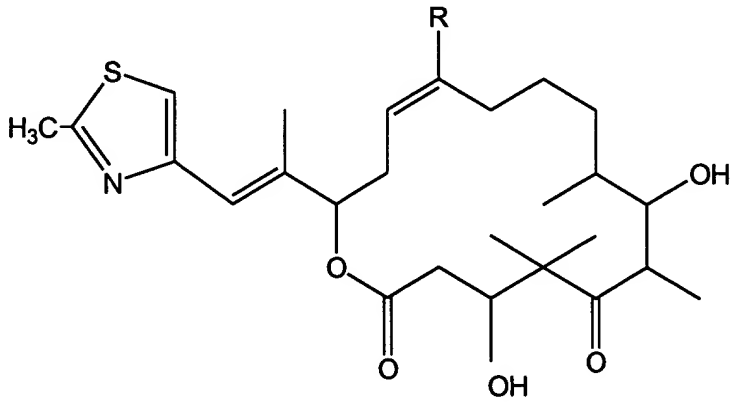
Claims 5-14 (Withdrawn and cancelled).

Claim 15. (Amended) A composition for plant protection in agriculture, forestry or horticulture comprising a compound according to claim 20 in combination with at least one carrier or diluent.

Claim 16. (Amended) A therapeutic composition comprising a compound according to claim 20 in which R is hydrogen in combination with a pharmaceutically acceptable carrier or diluent.

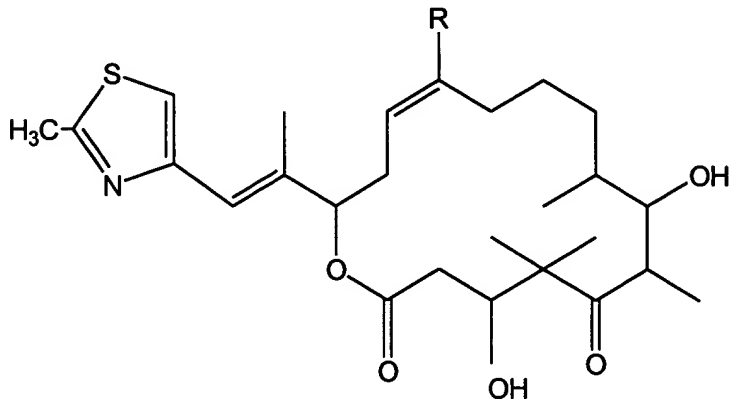
Claim 17. (Cancelled)

Claim 18. (Amended) A therapeutic composition comprising a compound of the formula



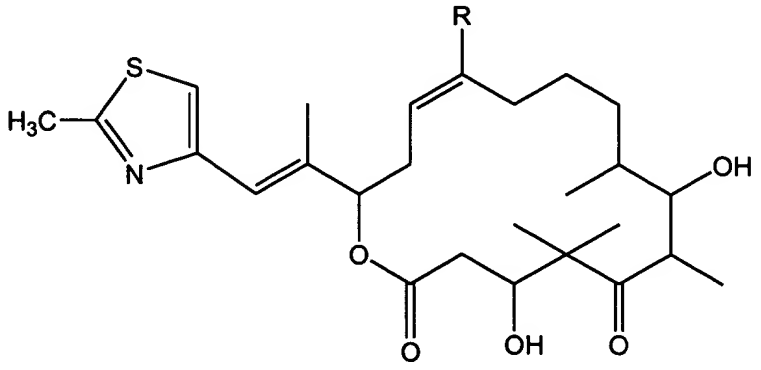
wherein R is hydrogen or methyl in combination with a pharmaceutically acceptable carrier or diluent.

Claim 19. (Amended) A method of treating malignant tumors comprising administering to a recipient in need thereof a therapeutic amount of a compound of the formula



wherein R is hydrogen or methyl.

Claim 20. (Amended) A compound of the formula:



in which R is hydrogen or methyl, said compound having a state of purity such as to be substantially free of other major metabolic products produced by *Sorangium cellulosum.*

Claim 21. (New) A therapeutic composition comprising a compound according to claim 20 in which R is methyl in combination with a pharmaceutically acceptable carrier or diluent.

* * *

REMARKS

Applicants wish to express their appreciation to Examiner Solola for the courtesy of an interview with their undersigned attorney, Bruce M. Collins, on May 18, 2004. At that time, Applicants' previously submitted Revised Request for Interference under 37 CFR §1.607, the Danishefsky patents and reissue application, Applicants' application and priority document, and Applicants' claims were analyzed in detail, as is now more fully discussed in the following comments and confirmation of that interview.

Applicants' Claims

During the interview, the Examiner reviewed Applicants' claims for compliance with 35 U.S.C. § 112, 2nd paragraph, and raised several formal matters.

In particular, claims 1 and 2 on the one hand and claims 3 and 4 on the other were deemed to be redundant as written. Clarification was also requested as to their relationship to claim 20.

Claim 20 is generic to claims 1-4 in that it encompasses compounds in which R is hydrogen or methyl. Accordingly claims 1-4 have been amended to now be dependent, either directly or indirectly, on claim 20.

The term "isolated" in the broadest claim asserted, claim 20, has be replaced by language specifying that the compound has a state of purity such as to be substantially free of other major metabolic products produced by *Sorangium cellulosum.* This language, as with the previous language, has been utilized to distinguish over the unpurified product of nature, namely epothilone compounds produced by, and existing in, cultures of *Sorangium cellulosum.* Basis will be found throughout the specification, as for example at page 1, line 27, through page 2, line 7 {sub paragraphs "(f)" though "(h2)"} and Example 1.

Claims 2 and 4 have been amended so as to be dependent on claims 1 and 3, respectively, and specify that the individual compound is sufficiently purified

to produce the 1H- and 13C-NMR spectra of Table 1 for epothilone C or epothilone D.

Claims 15 and 16 have been amended as also suggested by the Examiner to insert the grammatical article "A" and to convert the optional language concerning the carrier or diluent to the imperative; *e.g.*, both claims now require a carrier or diluent. Moreover, claim 16 has been limited to therapeutic compositions comprising epothilone C (R = H) while a new, parallel, claim 21 has been added to cover therapeutic compositions comprising epothilone D ($R = CH_3$). Claim 18, while independent, is generic in scope to claims 16 and 21 and again eliminates the optional language concerning the carrier or diluent.

Claim 19, directed to the method of use, has been amended to refer to the treatment of malignant tumors and to the administration of the compounds to a recipient in need thereof.

It is submitted that these amendments address all of the points discussed with the Examiner and that all claims now comply with 35 U.S.C. § 112, 2nd paragraph. It is respectfully requested that these be considered and appear in the event of issuance in the following order: 20, 1-4, 16, 21, 18, 19, and 15.

It is not believed that any additional fee is due for the present amendments but in the event a fee is deemed to be due, authority is hereby given to charge such deficiency to Deposit Account No. 13-2165.

With the claims now being limited to 1-4, 15, 16, and 18-21, the original inventorship has been reviewed and a requested amendment thereof under 37 CFR §1.48(b) is submitted herewith.

Applicants' Application and Priority Document

Other than the formal matters discussed above, no issue of patentability with regard to Applicants' application was raised during the interview.

At that time, however, Examiner Solola also examined the relevant passages in the translation of Applicants' priority document, specifically Formula 1

on page 1, Example 15 on pages 20-23,[1] the first paragraph on page 7, and original claim 13 on page 29, and confirmed that Applicants appear to be entitled to the benefit of German application No. 196 47 580.5 filed November 18, 1996 for claims 1-4, 15, 16, and 18-21.

Upon further review by Applicants subsequent to the interview, it was noted that the previously submitted certificate of translation inadvertently stated No. 196 47 580.5 was filed December 18, 1996 when in fact the document itself notes it was filed November 18, 1996. A corrected certificate of translation is submitted herewith.

Danishefsky's Patents/Application and Priority Claims

The patentability of the Danishefsky patent claims was not discussed in any detail. It was noted, however, that the disclosure cited by counsel for Danishefsky as purportedly providing support for broadened claim 23 of Danishefsky I *Reissue* application, namely col. 19, lines 1-27, does not in fact appear to provide support for the breadth of that claim. Specifically, col. 19, lines 1-27, does not describe R_1 being ethyl, propyl, hexyl, 2-(1,3-dioxolanyl)methyl, hydroxymethyl, or hydroxypropyl. Rather it discloses only hydrogen or methyl as permissible values. Consequently no description is apparent at col. 19, lines 1-27, for compounds falling within Danishefsky I Reissue claim 23 in which R_1 is ethyl, propyl, hexyl, 2-(1,3-dioxolanyl)methyl, hydroxymethyl, or hydroxypropyl and R' and R^0 is acetyl, benzyl, trialkylsilyl, dialkylarylsilyl, or alkyldiarylsilyl.

As best can be determined, none of the disclosures elsewhere in Danishefsky's specification provides descriptive support for the scope asserted in reissue claim 23. For example, the structure at col. 11, second formula, suffers in two respects: (*i*) it describes a different scope for R_1, namely "H, methyl, ethyl, n-propyl, n-butyl, n-hexyl, CH_2OH, or $(CH_2)_3OH$", and (*ii*) conversely it lacks the R' and R^0 variables recited in claim 23, namely acetyl, benzyl, trialkylsilyl, dialk-

[1] The reference to a second "Example 15" on page 24, while not addressed during the interview, obviously should read "Example 16".

ylarylsilyl, and alkyldiarylsilyl. Likewise, the structure shown at col. 7, first formula, has the same two deficiencies and moreover depicts a different structure than that of claim 23.

Concerning Danishefsky's claims of priority to some five provisional applications, none of these applications was before the Examiner at the time of the interview. Moreover, the record does not reflect which of these applications, if any, discloses (in accordance with 35 U.S.C. § 112, first paragraph) the subject matter presently claimed in Danishefsky I, Danishefsky I Reissue, and Danishefsky II.

Counsel for Applicants suggested to the Examiner that *if* a determination could not be made as to whether or not the claim of priority was justified, benefit should not be accorded.[2] First, the accordation of benefit implicitly suggests that descriptive support in the priority document has been affirmatively analyzed and affirmatively found to comply with 35 U.S.C. § 112. This clearly is not the case here, or at least was not the case as of the time of the interview. Second and even if Danishefsky were not given benefit, he would still be permitted to demonstrate {by preliminary motion under 37 CFR § 1.633(f)} that he in fact is entitled to the benefit of one or more of these provisional applications. This would simply mean that the burden of justifying such benefit falls on Danishefsky, not on the Examiner.

Relation of Applicants' Claims and Danishefsky Claims

During the interview, the Examiner asked counsel to assist him in tabulating the respective claims corresponding to the proposed count. While the resulting tabulation was retained by the Examiner, it is believed that the following, with one possible exception, parallels and accurately reflects that tabulation:

[2] Whether or not Danishefsky is accorded benefit will not alter the junior party/ senior party designations and thus would not effect the burden of proof.

	Danishefsky I 08/986,025	Danishefsky I Reissue 10/454,738	Danishefsky II 08/986,025	Reichenbach 09/313,524
Compounds	1-6	1-6, 23		1-4, 20
Pharmaceutical Compositions	7-13	7-13	1-2,	16,18, 21
Pharmaceutical Methods of Use	14-22	14-22	6-22, 27-30, 34-37	19

The disposition of Applicants' claim 15 during the interview is not recalled by counsel but upon reflection, it is submitted that claim 15 should not correspond to the count since it pertains to a different method than claim 19 (which would correspond to the count).

The Examiner and counsel also discussed the relationship of the parties' claims and the eventual count. Counsel suggested that under the current rules, there was no need for the claims of one party to correspond to the claims of the other party. Rather, the critical determination was whether the claims of each party corresponded to *the count*, a determination involving whether the various claims defined the same patentable invention under 37 CFR §1.601(n).

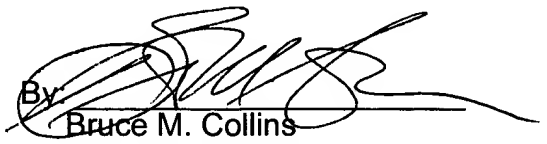
Notwithstanding the above tabulation, the Examiner agreed that in the present situation, the claims to compounds, pharmaceutical compositions and pharmaceutical methods of use should all be designated as corresponding to a single count. In this regard (*i*) none of the four parties and (*ii*) none of the various examiners has ever suggested these represented separate inventions that could justify the issuance of separate patents. For a more detailed analysis of why these all constitute a single patentable invention under 37 CFR § 1.601(n), the Examiner is respectfully referred to Applicants' analysis of these claims spanning pages 10-28 of the previously filed Revised Request for Interference under 37 CFR §1.607.

Information required for the PTO Form 850 is annexed hereto for the convenience of the Examiner. With the exception of the proposed count which remains the same, this information has been updated and revised on the basis of the points raised by the Examiner and discussed during the interview. This

form thus supersedes the information annexed to the previously filed Revised Request for Interference under 37 CFR §1.607.

Favorable reconsideration is earnestly solicited.

Respectfully Submitted,
MATHEWS, COLLINS, SHEPHERD & McKAY, P.A.
Attorneys for Applicants



By:
Bruce M. Collins
Reg. No. 20,066

June 10, 2004

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APPENDIX

Proposed Count Attached

PARTY	APPLICATION NO.	FILING DATE	PATENT NO.	ISSUE DATE
Reichenbach **(HOEFLE and STEINMETZ)**	09/313,524	May 17, 1999	N/A	N/A
Maintenance fees have been paid [ ] or are not due yet [ ]				
PROPOSED PRIORITY BENEFIT				
COUNTRY	APPLICATION NO.	FILING DATE	PATENT NO.. IF ANY	ISSUE DATE, IF ANY
Germany	196 47 580.5	Nov. 18, 1996		
The claim(s) of this party corresponding to this count				
PATENTED OR PATENTABLE PENDING CLAIMS **1-4, 16, 18-21**		UNPATENTABLE PENDING CLAIMS		
The claim(s) of this party NOT corresponding to this count:				
PATENTED OR PATENTABLE PENDING CLAIMS **15**		UNPATENTABLE PENDING CLAIMS		

PARTY	APPLICATION NO.	FILING DATE	PATENT NO.	ISSUE DATE
DANISHEFSKY	08/986,025	Dec. 3, 1997	6,242,469	Jun. 5, 2001
Maintenance fees have been paid [ ] or are not due yet [√]				
PROPOSED PRIORITY BENEFIT				
COUNTRY	APPLICATION NO.	FILING DATE	PATENT NO.. IF ANY	ISSUE DATE, IF ANY
The claim(s) of this party corresponding to this count				
PATENTED OR PATENTABLE PENDING CLAIMS **1-22**		UNPATENTABLE PENDING CLAIMS		
The claim(s) of this party NOT corresponding to this count:				
PATENTED OR PATENTABLE PENDING CLAIMS None		UNPATENTABLE PENDING CLAIMS		

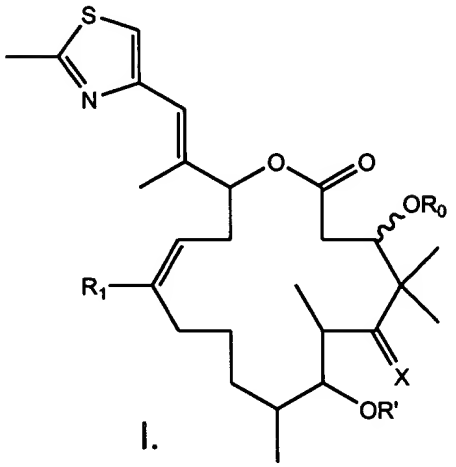
APPENDIX

PARTY	APPLICATION NO.	FILING DATE	PATENT NO.	ISSUE DATE
DANISHEFSKY	RE No. 10/454,738	Jun. 5, 2003	N/A	N/A
Maintenance fees have been paid [ ] or are not due yet [ ]				
PROPOSED PRIORITY BENEFIT				
COUNTRY	APPLICATION NO.	FILING DATE	PATENT NO.. IF ANY	ISSUE DATE, IF ANY
The claim(s) of this party corresponding to this count				
PATENTED OR PATENTABLE PENDING CLAIMS **1-22**		UNPATENTABLE PENDING CLAIMS **23**		
The claim(s) of this party NOT corresponding to this count:				
PATENTED OR PATENTABLE PENDING CLAIMS None		UNPATENTABLE PENDING CLAIMS		

PARTY	APPLICATION NO.	FILING DATE	PATENT NO.	ISSUE DATE
DANISHEFSKY	09/691,615	Oct. 18, 2000	6,284,781	Sep. 4, 2001
Maintenance fees have been paid [ ] or are not due yet [√]]				
PROPOSED PRIORITY BENEFIT				
COUNTRY	APPLICATION NO.	FILING DATE	PATENT NO.. IF ANY	ISSUE DATE, IF ANY
The claim(s) of this party corresponding to this count				
PATENTED OR PATENTABLE PENDING CLAIMS **1, 2, 5-23, 27-30, and 34-37**		UNPATENTABLE PENDING CLAIMS		
The claim(s) of this party NOT corresponding to this count:				
PATENTED OR PATENTABLE PENDING CLAIMS 3, 4, 24- 26, and 31 33		UNPATENTABLE PENDING CLAIMS		

Proposed Count

An isolated and purified compound of the formula:



I.

wherein

R_1 is hydrogen, methyl, ethyl, propyl, hexyl, 2-(1,3-dioxolanyl)methyl, hydroxymethyl, or hydroxypropyl,

X is O, and

R_0 and R' are independently hydrogen, acetyl, benzyl, trialkylsilyl, dialkylarylsilyl, or alkyldiarylsilyl; or

a pharmaceutical composition comprising an isolated and purified compound of formula I wherein R_1 is hydrogen, methyl, ethyl, propyl, hexyl, 2-(1,3-dioxolanyl)methyl, hydroxymethyl, or hydroxypropyl, X is O, and R_0 and R' are independently hydrogen or acetyl, in combination with a pharmaceutical carrier; or

a method of treating cancer in a subject suffering therefrom comprising administering to the subject a therapeutically effective amount of an isolated and purified compound of formula I wherein R_1 is hydrogen, methyl, ethyl, propyl, hexyl, 2-(1,3-dioxolanyl)methyl, hydroxymethyl, or hydroxypropyl, X is O, and R_0 and R'

are independently hydrogen or acetyl.

IN THE MATTER OF German Patent Applications in the name of Gesellschaft fuer Biotechnologische Forschung mbH (GBF) filed under 196 47 580.5

I, Dr. Hans D. Boeters, translator and patent attorney of BOETERS & BAUER, Bereiteranger 15, D-81541 München, Germany, do solemnly and sincerely declare that

1. I am conversant with the English and German languages and am a competent translator thereof.

2. I am the same Dr. Hans D. Boeters who on June 9, 1999 certified the translation of German application 196 47 580.5.

3. My June 9, 1999 certification stated 196 47 580.5 was filed on December 18, 1996 when in fact 196 47 580.5 was filed on November 18, 1996.

4. The attached is, to the best of my knowledge and belief, a true and correct translation of the patent application filed under 196 47 580.5 by Gesellschaft fuer Biotechnologische Forschung mbH (GBF) with the German Patent Office on November 18, 1996 for "Epothilons C and D, preparations and compositions" and the Official Certificate attached thereto.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that

such willful false statements may jeopardize the validity of U.S. Patent Application Serial No. 09/313,524 or any patent issued thereon.

Dr. Hans D. Boeters

Date: May 31, 2004

FEDERAL REPUBLIC OF GERMANY

CERTIFICATE

Gesellschaft für Biotechnologische Forschung mbH (GBF) of Braunschweig/Germany filed at the German Patent Office on 18th November, 1996 a Patent Application entitled

"Epothilons C and D, preparation and compositions".

The attached documents are a correct and accurate reproduction of the original supporting documents of this Patent Application.

The Application has provisionally been given in the German Patent Office the symbols C 07 D, C 07 F and A 01 N of the International Patent Classification.

Seal of the German
Patent Office

Munich, 18th December 1997
The President of the German
Patent Office
By order

(signature)

File reference: 196 47 580.5

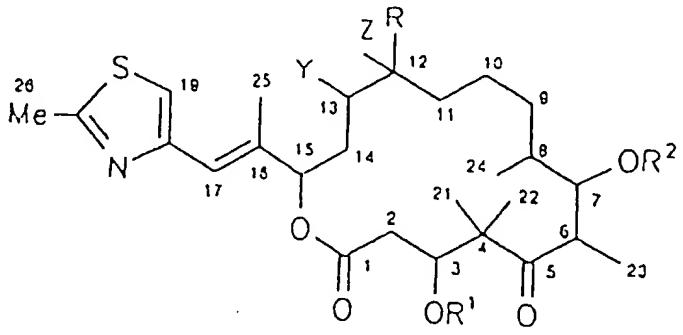
(Ebert)

18th November 1996/he

Our ref.: 8371-GBF

Epothilons C and D, preparation and compositions

The present invention relates generally to epothilon derivatives and to their use in the preparation of medicaments. The present invention relates especially to the preparation of epothilon derivatives of general formulae 1 to 7 shown hereinafter and to their use in the preparation of therapeutic compositions and compositions for plant protection.



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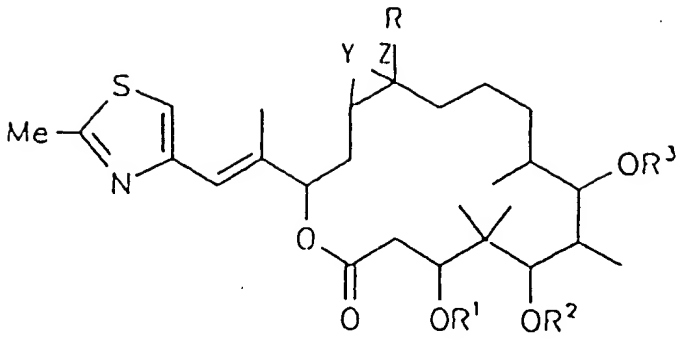

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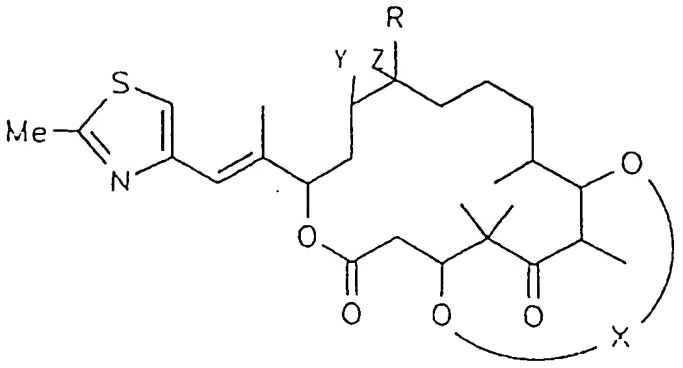

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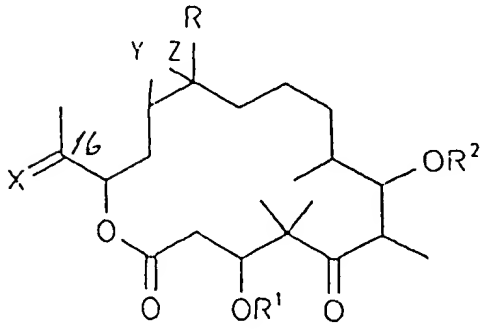

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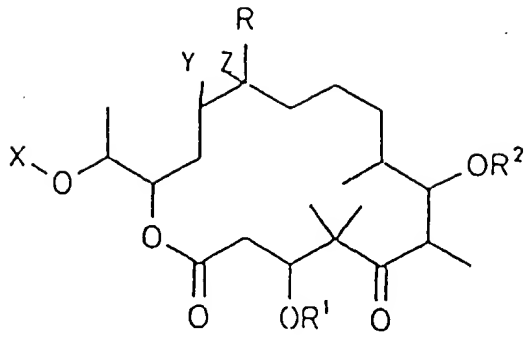

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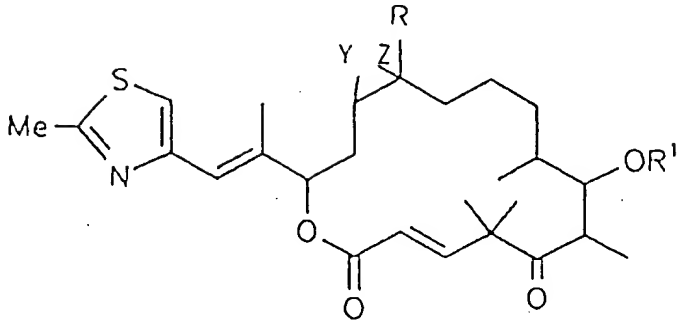

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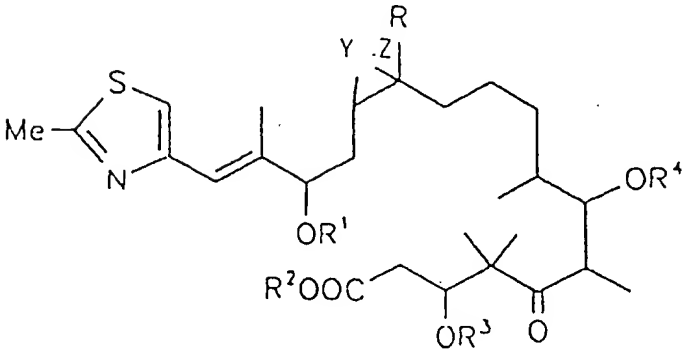

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In **formulae 1 to 7** given above:

R = H, C_{1-4}alkyl;

R^1, R^2, R^3, R^4, R^5 = H, C_{1-6}alkyl,
C_{1-6}acyl-benzoyl,
C_{1-4}trialkylsilyl,
benzyl,
phenyl,
benzyl or phenyl each substituted by C_{1-6}alkoxy,
C_6alkyl, hydroxy or by halogen;

it also being possible for two of the radicals R^1 to R^5 to occur together to form a group $-(CH_2)_n-$ wherein n is from 1 to 6, and the alkyl and acyl groups contained in the radicals are straight-chain or branched radicals;

Y and Z are either identical or different and each represents hydrogen, halogen, such as F, Cl, Br or I, pseudohalogen, such as -NCO, -NCS or $-N_3$, OH, O-(C_{1-6})acyl, O-(C_{1-6})alkyl, O-benzoyl. Y and Z may also be the O atom of an epoxy group, epothilon A and B not being claimed, or one of the C-C bonds of a C=C double bond.

In **formula 3**, X generally represents -C(O)-, -C(S)-, -S(O)-, $-CR^1R^2-$, wherein R^1 and R^2 are as defined above, or $-SiR_2-$ wherein R is as defined above.

In **formula 4**, X represents oxygen, NOR^3, $N-NR^4R^5$ or $N-NHCONR^4R^5$, wherein the radicals R^3 to R^5 are as defined above.

In **formula 5**, X represents hydrogen, C_{1-18}alkyl, C_{1-18}acyl, benzyl, benzoyl or cinnamoyl.

For epothilons A and B, see DE-A-41 38 042.

Compounds according to general **formula 1** can be obtained starting from epothilon A and B and from their 3-O- and/or 7-O-protected derivatives by opening the 12,13-epoxy group. If hydrohalic acids are used for that purpose in a preferably non-aqueous solvent, there being obtained the halohydrins X = Hal, Y = OH and Y = OH, Y = Hal. Protonic acids, such as, for example, toluenesulphonic acid and

trifluoroacetic acid, result, in the presence of water, in 12,13-diols which are then acylated (e.g. with carboxylic acid anhydrides and pyridine or triethylamine/DMAP) or alkylated (alkylhalides and silver oxide) according to standard processes. For that purpose, the 3- and 7-hydroxy groups may be protected temporarily in the form of a formate (removal with NH_3/MeOH) or of a p-methoxybenzyl ether (removal with DDQ).

Compounds according to general **formula 2** are obtainable from epothilon A and B and also from their 3-O- and/or 7-O-protected derivatives by reduction, for example with $NaBH_4$ in methanol. If 3-OH and/or 7-OH are protected reversibly, then after acylation or alkylation and removal of the protecting groups there may be obtained 5-O-monosubstituted or 3,5- or 5,7-O-disubstituted derivatives of general **formula 2**.

Reactions of epothilon A and B with bifunctional electrophilic reagents, such as (thio)phosgene, (thio)carbonyldiimidazole, thionyl chloride or dialkylsilyl dichlorides or bistriflates yield compounds of general **formula 3**. Pyridine, trialkylamines, optionally together with DMAP or 2,6-lutidine in an aprotic solvent serve as auxiliary bases in the process. The 3,7-acetals of general **formula 3** are produced by transacetalisation, for example of dimethylacetals in the presence of an acid catalyst.

Compounds according to general **formula 4** are obtained from epothilon A and B or from 3-O- and/or 7-O-protected derivatives thereof by ozonolysis and reductive working up, for example with dimethyl sulphide. The C-16-ketones may then be converted into oximes, hydrazones or semicarbazones in accordance with standard processes known to the person skilled in the art. They are, moreover, converted into C-16-/C-17-olefins by Wittig, Wittig-Horner, Julia or Petersen olefination.

The 16-hydroxy derivatives according to general **formula 5** are obtainable by reduction of the C-16-keto group, for example with an aluminium hydride or borohydride. If 3-OH and 7-OH are provided with suitable protecting groups, the 16-hydroxy derivatives may be either acylated or alkylated. The 3-OH and 7-OH groups are freed, for example, in the case of O-formyl by NH_3/MeOH and, in the case of O-p-methoxybenzyl, by DDQ.

The compounds of general **formula 6** are obtained from derivatives of epothilon A and B, in which the 7-OH group has been protected by acyl or ether groups, by, for example, formylating, mesylating or tosylating the 3-OH group and then eliminating it by treatment with a base, for example DBU. The 7-OH group can be freed as described above.

Compounds of general **formula 7** are obtained from epothilon A and B or from 3-OH- and 7-OH-protected derivatives thereof by basic hydrolysis, for example with NaOH in MeOH or MeOH/water. Preferably compounds of general **formula 7** are obtained from epothilon A or B or from 3-OH- or 7-OH-protected derivatives thereof by enzymatic hydrolysis, especially with esterases or lipases. The carboxy group can be converted to an ester with a diazoalkane after protection of the 19-OH group by alkylation.

Moreover, compounds of **formula 7** may be converted into compounds of **formula 2** by lactonisation in accordance with the methods of Yamaguchi (trichlorobenzoyl chloride/DMAP), Corey (aldrithiol/triphenylphosphine) or Kellogg (omega-bromic acid/caesium carbonate). Relevant working methods may be found in Inanaga *et al.* in Bull. Chem. Soc. Japan, 52 (1979) 1989; Corey & Nicolaou in J. Am. Chem. Soc., 96 (1974) 5614; and Kruizinga & Kellogg in J. Am. Chem. Soc., 103 (1981) 5183.

To prepare the compounds according to the invention, it is also possible to start from epothilon C or D, where, for the derivatisation, reference may be made to the derivatisation methods described above. The 12,13-double bond may be hydrogenated, for example catalytically or with diimine; or epoxidised, for example with dimethyldioxirane or with a peracid; or converted into a dihalide, dipseudohalide or diazide.

The invention relates also to compositions for plant protection in agriculture, forestry and/or horticulture, consisting of one or more of the above-mentioned epothilon derivatives or consisting of one or more of the above-mentioned epothilon derivatives together with one or more customary carrier(s) and/or diluent(s).

Finally, the invention relates to therapeutic compositions consisting of one or more of the above-mentioned compounds or of one or more of the above-mentioned compounds together with one or more customary carrier(s) and/or diluent(s). Those compositions may especially demonstrate cytotoxic activities and/or cause immunosuppression and/or be used to combat malignant tumours; they are especially preferably usable as cytostatic agents.

The invention is illustrated and described hereinafter in greater detail by the description of a number of selected embodiments.

Examples

Example 1:
Compound 1a

20 mg (0.041 mmol) of epothilon A are dissolved in 1 ml of acetone, 50 µl (0.649 mmol) of trifluoroacetic acid are added and the reaction mixture is stirred overnight at 50°C. The reaction mixture is worked up by adding 1M phosphate buffer pH 7 and extracting the aqueous phase four times with ethyl acetate. The combined organic phases are washed with saturated sodium chloride solution, dried over sodium sulphate and freed of solvent. The crude product is purified by preparative layer chromatography (eluant: dichloromethane/acetone, 85:15).

Yield: 4 mg (19%) of isomer I
4 mg (19%) of isomer II

Isomer I

R_f (dichloromethane/acetone, 85:15) : 0.46

IR (film): ny = 3440 (m, b, sh), 2946 (s, sh), 1734 (vs), 1686 (m), 1456 (m), 1375 (w), 1256 (s, sh), 1190 (w, b, sh), 1071 (m, sh), 884 (w), 735 (w) cm^{-1}.

MS (20/70 eV) : m/e (%) = 493 (43 $[M-H_2O]^+$), 394 (47), 306 (32), 206 (30), 181 (40), 166 (72), 139 (100), 113 (19), 71 (19), 57 (24), 43 (24).

Microanalysis: $C_{26}H_{39}O_6NS$ calc.: 493.2498 for $[M-H_2O]^+$
found: 493.2478

Isomer II

R_f (dichloromethane/acetone, 85:15) : 0.22

IR (film) : ny = 3484 (s, b, sh), 2942 (vs, sh), 1727 (vs), 1570 (w), 1456 (m), 1380 (m), 1265 (s), 1190 (w), 1069 (m), 975 (w) cm^{-1}.

MS (20/70 eV) : m/e (%) = 493 (21 $[M-H_2O]^+$), 394 (12), 306 (46), 206 (37), 181 (63), 166 (99), 139 (100), 113 (21), 71 (23), 57 (33), 43 (28).

Microanalysis: $C_{26}H_{39}O_6NS$ calc. : 493.2498 for $[M-H_2O]^+$
found: 493.2475

Example 2:

Compound 1b

55 mg (0.111 mmol) of epothilon A are dissolved in 0.5 ml of tetrahydrofuran, 0.S ml of 1N hydrochloric acid is added, and the reaction mixture is stirred at room temperature for 30 minutes. 1N Phosphate buffer pH 7 is then added and the aqueous phase is extracted four times with ethyl acetate. The combined organic phases are washed with saturated sodium chloride solution, dried over sodium sulphate and freed of solvent. The crude product is purified by preparative layer chromatography (eluant: dichloromethane/methanol, 90:10). Yield: 19 mg (32%).

R_f (dichloromethane/methanol, 90:10) : 0.46

IR (film) : ny = 3441 (s, br, sh), 2948 (s, sh), 1725 (vs, sh), 1462 (m), 1381 (w), 1265 (m), 1154 (w), 972 (m, br, sh) cm^{-1}.

UV (methanol) : $lambda_{max}$ (lg epsilon) = 210 (4.29), 248 (4.11) nm.

MS (20/70 eV) : m/e (%) = 529 (13 [M^+]), 494 (10), 342 (38), 306 (23), 194 (32), 164 (100), 140 (31), 113 (15), 57 (16).

Microanalysis: $C_{26}H_{40}O_6ClNS$ calc.: 529.2265 for [M^+], found: 529.2280

Example 3:

Compound 1c

25 mg (0.047 mmol) of 12-chloro-13-hydroxy-epothilon A (**1b**) are dissolved in 1 ml of dichloromethane, and 29 mg (0.235 mmol) of dimethylaminopyridine, 151 µl (1.081 mmol) of triethylamine and 20 µl (0.517 mmol) of 98% formic acid are added. The reaction mixture is cooled with ice/salt. When -15°C has been reached, 40 µl (0.423 mmol) of acetic anhydride are added to the reaction mixture, which is stirred for 70 minutes at -15°C. Since thin-layer chromatography shows that the reaction is not complete, a further 6 mg (0.047 mmol) of dimethylaminopyridine, 7 µl (0.047 mmol) of triethylamine, 2 µl of 98% formic acid (0.047 mmol) and 4 µl (0.047 mmol) of acetic anhydride are added to the reaction mixture, which is stirred for 60 minutes. The reaction mixture is worked up by heating to room temperature, adding 1M phosphate buffer pH 7 and extracting the aqueous phase four times with ethyl acetate. The combined organic phases are washed with saturated sodium chloride solution, dried over sodium sulphate and freed of solvent. The crude product is purified by preparative layer chromatography (eluant: dichloromethane/acetone, 90:10).
Yield: 5 mg (18%).

R_f (dichloromethane/acetone, 90:10) : 0.67

IR (film) : ny = 3497 (w, b, sh), 2940 (s, b, sh), 1725 (vs), 1468 (m, b, sh), 1379 (m), 1265 (s), 1253 (s), 1175 (vs), 972 (m, b, sh), 737 (s) cm^{-1}.

MS (20/70 eV) : m/e (%) = 613 (9 [M^+]), 567 (43), 472 (63), 382 (23), 352 (21), 164 (100), 151 (33), 96 (31), 69 (17), 44 (26).

Microanalysis: $C_{29}H_{40}O_9NSCl$ calc.: 613.2112 for [M^+]
found: 613.2131

Example 4:

Compound 1d

10 mg (0.020 mmol) of epothilon B are dissolved in 0.5 ml of tetrahydrofuran, 0.5 ml of 1N hydrochloric acid is added and the reaction mixture is stirred at room temperature for 30 minutes. 1M Phosphate buffer pH 7 is then added and the aqueous phase is extracted four times with ethyl acetate. The combined organic phases are washed with saturated sodium chloride solution, dried over sodium sulphate and freed of solvent. The crude product is purified by preparative layer chromatography (eluant: dichloromethane/acetone, 85:15).
Yield: 1 mg (9%).

R_f (dichloromethane/acetone, 85:15) : 0.38

MS (20/70 eV) : m/e (%) = 543 (3 [M^+]), 507 (14), 320 (19), 234 (9), 194 (17), 182 (23), 164 (100), 140 (22), 113 (14), 71 (13).

Microanalysis: $C_{27}H_{42}O_6NSCl$ calc. : 543.2421 for [M^+]
found: 543.2405

Example 5:

Compound 2a

100 mg (0.203 mmol) of epothilon A are dissolved in 4 ml of tetrahydrofuran/-1M phosphate buffer pH 7 (1:1), and sodium borohydride (150 mg = 3.965 mmol) is added until the starting material has reacted completely according to thin-layer chromatography. Dilution with 1M phosphate buffer pH 7 is then carried out and the aqueous phase is extracted four times with ethyl acetate. The combined organic phases are washed with saturated sodium chloride solution, dried over sodium sulphate and freed of solvent. The crude product is purified by silica chromatography (eluant: dichloromethane/acetone, 95:5 - gradient - to dichloromethane/acetone, 85:15).

Yield: (20%)

R_f (dichloromethane/acetone, 75:25) : 0.27

IR (film) : ny = 3413 (s, b, sh), 2965 (vs, sh), 1734 (vs), 1458 (m, b, sh), 1383 (m, sh), 1264 (s, b, sh), 1184 (m, b, sh), 1059 (s, sh), 966 (s), 885 (w), 737 (m) cm^{-1}.

MS (20/70 eV) : m/e (%) = 495 (6 [M^+]), 477 (8), 452 (12), 394 (9), 364 (16), 306 (49), 194 (19), 178 (35), 164 (100), 140 (40), 83 (21), 55 (27).

Microanalysis : $C_{26}H_{41}O_6NS$ calc. : 495.2655 for [M^+]
found: 495.2623

Example 6:

Compound 3a-d (a-d are stereoisomers)

100 mg (0.203 mmol) of epothilon are dissolved in 3 ml of pyridine, 50 µl (0.686 mmol) of thionyl chloride are added and the reaction mixture is stirred at room temperature for 15 minutes. 1M Phosphate buffer pH 7 is then added and the aqueous phase is extracted four times with ethyl acetate. The combined organic phases are washed with saturated sodium chloride solution, dried over sodium sulphate and freed of solvent. The crude product is purified and the four stereoisomers **3a-d** are separated by preparative layer chromatography (eluant: toluene/methanol, 90:10).

Compound 3a

Yield : 4 mg (12%)

R_f (toluene/methanol, 90:10) : 0.50

IR (film) : ny = 2961 (m, b, sh), 1742 (vs), 1701 (vs), 1465 (m, sh), 1389 (m, sh), 1238 (s, sh), 1210 (vs, sh), 1011 (s, sh), 957 (s, b, sh), 808 (m, sh), 768 (s, sh) cm^{-1}.

UV (methanol) : $lambda_{max}$ (lg epsilon) = 210 (4.50), 248 (4.35) nm.

MS (20/70 eV) : m/e (%) = 539 (40 [M^+]), 457 (22), 362 (16), 316 (27), 222 (30), 178 (30), 164 (100), 151 (43), 96 (38), 69 (29), 55 (28), 43 (20).

Microanalysis: $C_{26}H_{37}O_7NS_2$ calc. : 539.2011 for [M^+]

Compound 3b

Yield : 14 mg (13%)

R_f (toluene/methanol, 90:10) : 0.44

IR (film) : ny = 2963 (s, br, sh), 1740 (vs), 1703 (s), 1510 (w), 1464 (m, br, sh), 1389 (m, sh), 1240 (s, br, sh), 1142 (m), 1076 (w), 1037 (w), 1003 (m), 945 (s, br, sh), 806 (m, sh), 775 (s), 737 (m) cm^{-1}.

UV (methanol) : $lambda_{max}$ (lg epsilon) = 211 (4.16), 250 (4.08) nm.

MS (20/70 eV) : m/e (%) = 539 (27 [M^+]), 475 (17), 322 (41), 306 (67), 222 (16), 206 (17), 194 (19), 178 (32), 164 (100), 151 (33), 125 (18), 113 (15), 96 (39), 81 (23), 64 (58), 57 (42), 41 (19).

Microanalysis : $C_{26}H_{37}O_7NS_2$ calc. : 539.2011 for [M^+]
found: 539.1998

Compound 3c

Yield : 4 mg (4%)

R_f (toluene/methanol, 90:10) : 0.38

MS (20/70 eV) : m/e (%) = 539 (51 [M^+]), 322 (22), 306 (53), 222 (36), 178 (31), 164 (100), 151 (41), 96 (25), 81 (20), 69 (26), 55 (25), 41 (25).

Microanalysis : $C_{26}H_{37}O_7NS_2$ calc. : 539.2011 for [M^+]
found: 539.2001

Compound 3d

Yield : 1 mg (1%)

R_f (toluene/methanol, 90:10) : 0.33

MS (20/70 eV) : m/e (%) = 539 (69 [M^+]), 322 (35), 306 (51), 222 (41), 178 (31), 164 (100), 151 (46), 96 (31), 81 (26), 69 (34), 55 (33), 41 (35)

Microanalysis : $C_{26}H_{37}O_7NS_2$ calc. : 539.2011 for [M^+]
found: 539.1997

Example 7

Compound 4a

10 mg (0.020 mmol) of epothilon A are dissolved in 2 ml of dichloromethane, cooled to -70°C and then treated with ozone for 5 minutes until there is a slight blue coloration. 0.5 ml of dimethyl sulphide is subsequently added to the resulting reaction mixture, which is heated to room temperature. The reaction mixture is worked up by freeing it of solvent and finally by preparative layer chromatography (eluant: dichloromethane/-acetone/methanol, 85:10:5).

Yield : 5 mg (64%)

R_f (dichloromethane/acetone/methanol, 85:10:5) : 0.61

IR (film) : ny = 3468 (s, br, sh), 2947 (s, br, sh), 1734 (vs, sh), 1458 (w), 1380 (w), 1267 (w), 1157 (w), 1080 (w), 982 (w) cm^{-1}.

UV (methanol) : $lambda_{max}$ (lg epsilon) = 202 (3.53) nm.

MS (20/70 eV) : m/e (%) = 398 (2 [M^+]), 380 (4), 267 (14), 249 (17), 211 (20), 193 (26), 171 (34), 139 (34), 111 (40), 96 (100), 71 (48), 43 (50).

Microanalysis : $C_{21}H_{34}O_7$: calc. : 398.2305 for [M^+]
found: 398.2295

Example 8:
Compound 6a

10 mg (0.018 mmol) of 3,7-di-O-formyl-epothilon A are dissolved in 1 ml of dichloromethane, 27 µl (0.180 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) are added and the reaction mixture is stirred at room temperature for 60 minutes.
The reaction mixture is worked up by adding 1M sodium dihydrogen phosphate buffer pH 4.5 and extracting the aqueous phase four times with ethyl acetate. The combined organic phases are washed with saturated sodium chloride solution, dried over sodium sulphate and freed of solvent. After the solvent has been removed, the resulting crude product is dissolved in 1 ml of methanol, 200 µl of an ammoniacal methanol solution (2 mmol NH_3/ml methanol) are added and the mixture is stirred overnight at room temperature. To work up, the solvent is removed *in vacuo*.

Yield : 4 mg (22%)

R_f (dichloromethane/acetone, 85:15) : 0.46

IR (film) : ny = 3445 (w, br, sh), 2950 (vs, br, sh), 1717 (vs, sh), 1644 (w), 1466 (m, sh), 1370 (m, sh), 1267 (s, br, sh), 1179 (s, sh), 984 (s, sh), 860 (w), 733 (m) cm^{-1}.

UV (methanol) : $lambda_{max}$ (lg epsilon) = 210 (4.16) nm.

MS (20/70 eV) : m/e (%) = 475 (28 [M^+]), 380 (21), 322 (37), 318 (40), 304 (66), 178 (31), 166 (100), 151 (29), 140 (19), 96 (38), 81 (20), 57 (26).

Microanalysis : $C_{26}H_{37}O_5NS$ calc.: 475.2392 for [M^+]
found: 475.2384

Example 9:
Compound 6b

50 mg (0.091 mmol) of 3,7-di-O-formyl-epothilon A are dissolved in 1 ml of dichloroethane, 2 ml (0.013 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) are added and the reaction mixture is stirred for 12 hours at 90°C.
The reaction mixture is worked up by adding 1M sodium dihydrogen phosphate buffer pH 4.5 and extracting the aqueous phase four times with ethyl acetate. The combined organic phases are washed with saturated sodium chloride solution, dried over sodium sulphate and freed of solvent. The crude product, consisting of two compounds, is purified by preparative layer chromatography (eluant: dichloro-methane/acetone, 90:10).

Yield : 7 mg (15%)

Substance code

R_f (dichloromethane/acetone, 90:10) : 0.62

IR (film) : ny = 2951 (m, br, sh), 1723 (vs), 1644 (w, br, sh), 1468 (w), 1377 (w), 1271 (m, br, sh), 1179 (s), 987 (m, br, sh), 735 (w, br, sh) cm^{-1}.

UV (methanol) : $lambda_{max}$ (lg epsilon) = 210 (4.44) nm.

MS (20/70 eV) : m/e (%) = 503 (68 [M^+]), 408 (58), 390 (32), 334 (25), 316 (34), 220 (21), 206 (27), 194 (20), 181 (33), 164 (100), 151 (34), 139 (28), 113 (20), 96 (82), 81 (33), 67 (24), 55 (26), 43 (22).

Microanalysis : $C_{27}H_{37}O_6NS$ calc.: 503.2342 for [M^+]
found: 503.2303

Example 10

Compound 6c

5 mg (0.009 mmol) of 3,7-di-O-acetyl-epothilon are dissolved in 1 ml of methanol, 150 µl of an ammoniacal methanol solution (2 mmol NH_3/ml methanol) are added and the reaction mixture is stirred overnight at 50°C.
To work up, the solvent is removed *in vacuo*. The crude product is purified by preparative layer chromatography (eluant: toluene/methanol, 90:10).

Yield : 3 mg (67%)

R_f (dichloromethane/acetone, 90:10) : 0.55

IR (film) : ny = 2934 (s, b, sh), 1719 (vs, b, sh), 1641 (m), 1460 (m, sh), 1372 (s, sh), 1237 (vs, b, sh), 1179 (s, sh), 1020 (s), 963 (s, sh), 737 (vs) cm^{-1}.

UV (methanol) : $lambda_{max}$ (lg epsilon) = 210 (4.33) nm.

MS (20/70 eV) : m/e (%) = 517 (57 [M^+]), 422 (58), 318 (31), 194 (20), 181 (34), 166 (100), 151 (31), 96 (96), 81 (32), 69 (27), 55 (29), 43 (69).

Microanalysis : $C_{28}H_{39}O_6NS$ calc.: 517.2498 for [M^+]
found: 517.2492

Example 11

Compound 7a

20 mg (0.041 mmol) of epothilon are dissolved in 0.5 ml of methanol, 0.5 ml of 1N sodium hydroxide solution is added and the reaction mixture is stirred at room temperature for 5 minutes.

The reaction mixture is worked up by adding 1M phosphate buffer pH 7 and extracting the aqueous phase four times with ethyl acetate. The combined organic phases are washed with saturated sodium chloride solution, dried over sodium sulphate and freed of solvent. The crude product is purified by preparative layer chromatography (eluant: dichloromethane/methanol, 85:15).

Yield :11 mg (52%)

R_f (dichloromethane/methanol, 85:15) : 0.92

IR (film) : ny = 3438 (s, br, sh), 2971 (vs, br, sh), 1703 (vs), 1507 (m), 1460 (s, sh), 1383 (m, sh), 1254 (w), 1190 (w, br, sh), 1011 (w, br, sh), 866 (w, br), 729 (s) cm^{-1}.

MS (20/70 eV) : m/e (%) = 423 (0.1 [M^+]), 323 (4), 168 (89), 140 (100), 85 (31), 57 (67).

Microanalysis : $C_{23}H_{37}O_4NS$ calc.: 423.2443 for [M^+]
found: 423.2410

Example 12:

Compound 7b

5 mg (0.009 mmol) of 7-O-acetyl-epothilon are dissolved in 1 ml of methanol, 200 µl of an ammoniacal methanol solution (2 mmol NH_3/ml methanol) are added and the reaction mixture is stirred at 50°C for two days. To work up, the solvent is removed *in vacuo*. The crude product is purified by preparative layer chromatography (eluant: toluene/methanol, 90:10).

Yield : 3 mg (59%)

R_f (dichloromethane/methanol, 90:10) : 0.63

IR (film) : ny = 3441 (m, b, sh), 2946 (s, sh), 1732 (vs), 1600 (w), 1451 (m), 1375 (m), 1246 (s, b, sh), 1013 (m, b, sh) cm^{-1}.

UV (methanol) : $lambda_{max}$ (lg epsilon) = 211 (3.75), 247 (3.59) nm.

MS (20/70 eV) : m/e (%) = 567 (1 [M^+]), 465 (4), 422 (7), 388 (5), 194 (5), 182 (7), 168 (65), 164 (17), 140 (100), 97 (10), 71 (22), 43 (27).

Microanalysis : $C_{29}H_{45}O_8NS$ calc.: 567.2866 for [M^+]
found: 567.2849

Example 13:

50 mg of epothilon A are dissolved in 20 µl of dimethyl sulphoxide and diluted with 30 ml of phosphate buffer (pH 7.1, 30 mM). After the addition of 5 mg of pig liver esterase (Boehringer Mannheim), the mixture is stirred for 2 days at 30°C. The mixture is acidified to pH 5 with 2N HCl and the epothilonic acid 7 is extracted with

ethyl acetate. The organic phase is dried with sodium sulphate and concentrated to dryness by evaporation *in vacuo*. Yield 48 mg (96%).

Example 14:

48 mg of epothilonic acid 7 are dissolved in 6 ml of abs. THF and, with stirring, 40 µl of triethylamine and 16 µl of 2,4,6-trichlorobenzoyl chloride are added. After 15 minutes, the precipitate is removed by filtration and the filtrate is added dropwise, within a period of 15 minutes, with rapid stirring, to a boiling solution of 20 mg of 4-dimethylaminopyridine in 200 ml of abs. toluene. After a further 10 minutes, the mixture is concentrated by evaporation *in vacuo* and the residue is partitioned between ethyl acetate/citrate buffer (pH 4). After separation by preparative HPLC, the evaporation residue of the organic phase yields 15 mg of epothilon A.

Example 15:
Epothilons C and D as starting materials

A. Production strain and culture conditions corresponding to the epothilon basic patent.

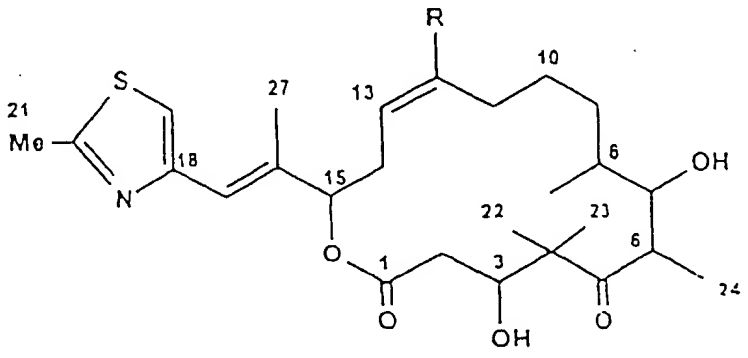
B. Production using DSM 6773

75 litres of culture are cultured as described in the basic patent and are used for inoculation into a production fermenter containing 700 litres of production medium consisting of 0.8% starch, 0.2% glucose, 0.2% soya flour, 0.2% yeast extract, 0.1% $CaCl_2 \times 2H_2O$, 0.1% $MgSO_4 \times 7H_2O$, 8 mg/litre of Fe-EDTA, pH = 7.4 and optionally 15 litres of Amberlite XAD-16 adsorber resin. Fermentation lasts for from 7 to 10 days at 30°C, with aeration with 2 m^3 air/h. The pO_2 is maintained at 30% by regulating the speed.

C. Isolation

The adsorber resin is separated from the culture using a 0.7 m^2 100-mesh process filter and is freed of polar impurities by washing with 3 bed volumes of water/methanol 2:1. Elution with 4 bed volumes of methanol yields a crude extract which is concentrated by evaporation *in vacuo* until the aqueous phase occurs. That is then extracted three times with the same volume of ethyl acetate. Concentration of the organic phase by evaporation yields 240 g of crude extract which is partitioned between methanol and heptane in order to separate off lipophilic impurities. From the methanolic phase there are obtained by concentration by evaporation *in vacuo* 180 g of isolate which is fractionated in three portions over Sephadex LH-20 (20 x 100 cm column, 20 ml/min methanol). The epothilons are contained in the fraction which is eluted in the retention time from 240 to 300 minutes and which comprises a total of 72 g. To separate the epothilons, chromatography is carried out in three portions on Lichrosorb RP-18 (15 µm, 10 x 40 cm column, eluant 180 ml/min methanol/water 65:35). After epothilon A and B there are eluted epothilon C at R_t = 90-95 min and epothilon D at R_t=100-110 min, which are obtained, after concentration by evaporation *in vacuo*, in each case in a yield of 0.3 g of a colourless oil.

D. Physical properties



Epothilon C R = H

Epothilon D R = CH_3

Epothilon C

$C_{26}H_{39}NO_5S$ [477]

ESI-MS : (positive ions) : 478.5 for $[M+H]^+$

1H and 13C, see NMR table

TLC: R_f = 0.82

TLC aluminium foil 60 F 254 Merck, eluant : dichloromethane/methanol = 9:1

Detection : UV extinction at 254 nm. Spraying with vanillin/sulphuric acid reagent, blue-grey coloration on heating to 120°C.

HPLC : R_t = 11.5 min

Column: Nucleosil 100 C-18 7 µm, 125 x 4 mm.

Eluant: methanol/water = 65:35

Flow rate : 1 ml/min

Detection: diode array

Epothilon D

$C_{27}H_{41}NO_5S$ [491]

ESI-MS : (positive ions) : 492.5 for $[M+H]^+$

1H and 13C, see NMR table

TLC : R_f = 0.82

TLC aluminium foil 60 F 254 Merck, eluant : dichloromethane/methanol = 9:1

Detection : UV extinction at 254 nm. Spraying with vanillin/sulphuric acid reagent, blue-grey coloration on heating to 120°C.

HPLC : R_t = 15.3 min

Column : Nucleosil 100 C-18 7 µm, 125 x 4 mm

Eluant : methanol/water = 65:35

Flow rate : 1 ml/min

Detection: diode array

Table: ^{1}H and ^{13}C NMR data of epothilon C and epothilon D in [D_6]DMSO at 300 MHz

	Epothilon C			Epothilon D		
H atom	δ (ppm)	C atom	δ (ppm)	δ (ppm)	C atom	δ (ppm)
		1	170.3		1	170.1
2-Ha	2.38	2	38.4	2.35	2	39.0
2-Hb	2.50	3	71.2	2.38	3	70.8
3-H	3.97	4	53.1	4.10	4	53.2
3-OH	5.12	5	217.1	5.08	5	217.4
6-H	3.07	6	45.4	3.11	6	44.4
7-H	3.49	7	75.9	3.48	7	75.5
7-OH	4.46	8	35.4	4.46	8	36.3
8-H	1.34	9	27.6	1.29	9	29.9
9-Ha	1.15	10	30.0	1.14	10	25.9
9-Hb	1.40	11	27.6	1.38	11	31.8*
10-Ha	1.15*	12	124.6	1.14*	12	138.3
10-Hb	1.35*	13	133.1	1.35*	13	120.3
11-Ha	1.90	14	31.1	1.75	14	31.6*
11-Hb	2.18	15	76.3	2.10	15	76.6
12-H	5.38**	16	137.3		16	137.2
13-H	5.44**	17	119.1	5.08	17	119.2
14-Ha	2.35	18	152.1	2.30	18	152.1
14-Hb	2.70	19	117.7	2.65	19	117.7
15-H	5.27	20	164.2	5.29	20	164.3
17-H	6.50	21	18.8	6.51	21	18.9
19-H	7.35	22	20.8	7.35	22	19.7
21-H_3	2.65	23	22.6	2.65	23	22.5
22-H_3	0.94	24	16.7	0.90	24	16.4
23-H_3	1.21	25	18.4	1.19	25	18.4
24-H_3	1.06	27	14.2	1.07	26	22.9
25-H_3	0.90			0.91	27	14.1
26-H_3				1.63		
27-H_3	2.10			2.11		

*, ** allocation interchangeable

Example 15:

Epothilon A and 12,13-bisepi-epothilon A from epothilon C

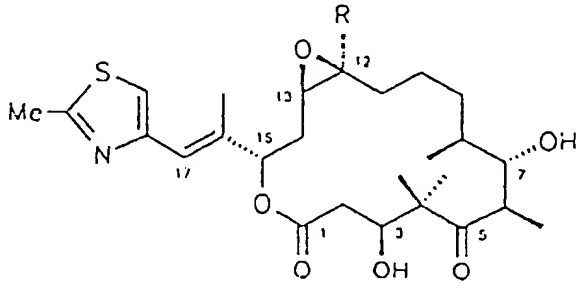
50 mg of epothilon A are dissolved in 1.5 ml of acetone, and 1.5 ml of a 0.07M solution of dimethyldioxirane in acetone are added. After 6 hours' standing at room temperature, concentration by evaporation *in vacuo* is carried out and separation is effected by preparative HPLC on silica gel (eluant: methyl tert-butyl ether/petroleum ether/methanol 33:66:1).

Yield:

25 mg of epothilon A, R_t = 3.5 min (analyt. HPLC, 7 µm, 4 x 250 mm column, eluant see above, flow rate 1.5 ml/min

and

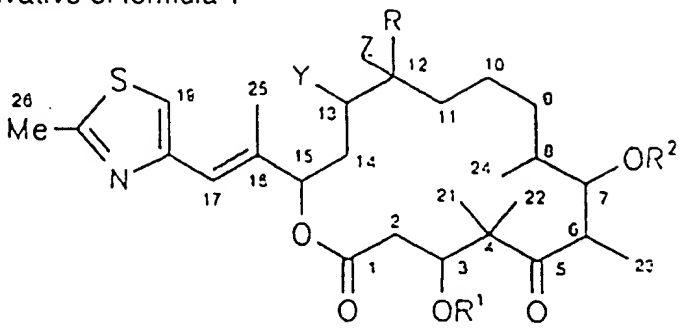
20 mg of 12,13-bisepi-epothilon A, R_t = 3.7 min, ESI-MS (pos. ions)
m/z = 494 $[M+H]^+$
^{1}H-NMR in $[D_4]$ methanol, selected signals:
delta = 4.32 (3-H), 3.79 (7-H), 3.06 (12-H), 3.16 (13-H), 5.54 (15-H), 6.69 (17-H), 1.20 (22-H), 1.45 (23-H).



12,13-bisepi-epothilon A R = H

Patent Claims

1. Epothilon derivative of formula 1

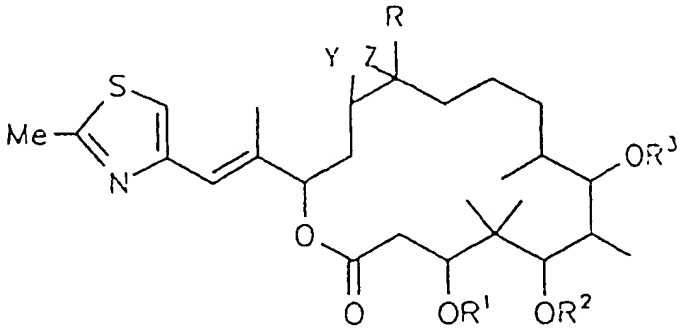


1

wherein

R = H, C_{1-4}alkyl; R^1, R^2 = H, C_{1-6}alkyl, C_{1-6}acyl, benzoyl, C_{1-4}trialkylsilyl, benzyl, phenyl, or benzyl or phenyl each substituted by C_{1-6}alkoxy, C_6alkyl, hydroxy or by halogen; and the alkyl and acyl groups contained in the radicals are straight-chain or branched radicals, and Y and Z are either identical or different and each represents hydrogen, halogen, pseudohalogen, OH, O-(C_{1-6})acyl, O-(C_{1-6})alkyl or O-benzoyl, or together form the O atom of an epoxy group or one of the C-C bonds of a C=C double bond, epothilon A and B being excluded.

2. Epothilon derivative of formula 2



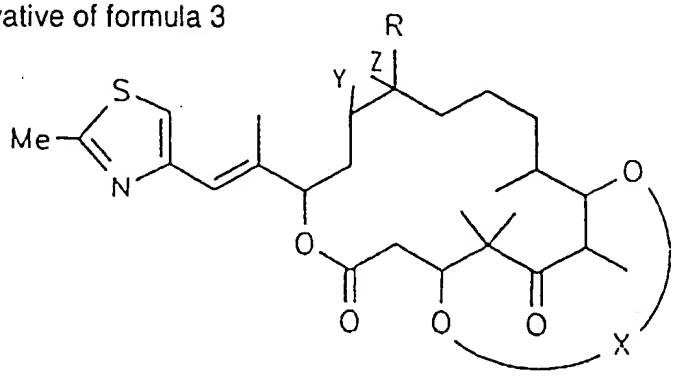
2

wherein

R = H, C_{1-4}alkyl; R^1, R^2, R^3 = H, C_{1-6}alkyl, C_{1-6}acyl, benzoyl, C_{1-4}trialkylsilyl, benzyl, phenyl, or benzyl or phenyl each substituted by C_{1-6}alkoxy, C_6alkyl, hydroxy or by

halogen; the alkyl and acyl groups contained in the radicals are straight-chain or branched radicals; and Y and Z are as defined according to claim 1.

3. Epothilon derivative of formula 3

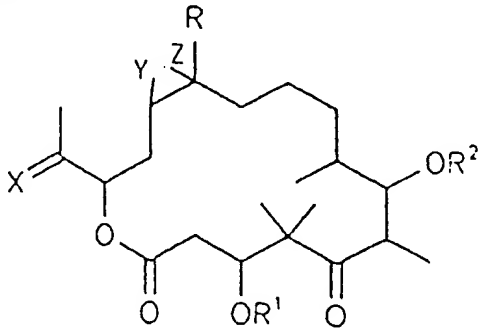


3

wherein

R = H, C_{1-4}alkyl; R^1, R^2 = H, C_{1-6}alkyl, C_{1-6}acyl, benzoyl, C_{1-4}trialkylsilyl, benzyl, phenyl, or benzyl or phenyl each substituted by C_{1-6}alkoxy, C_6alkyl, hydroxy or by halogen; the alkyl and acyl groups contained in the radicals are straight-chain or branched radicals, and X generally represents -C(O)-, -C(S)-, -S(O)-, $-CR^1R^2-$ or $-SiR_2-$, wherein R, R^1 and R^2 are as defined above and R^1 and R^2 may also together form an alkylene group having from 2 to 6 carbon atoms; and Y and Z are as defined according to claim 1.

4. Epothilon derivative of formula 4



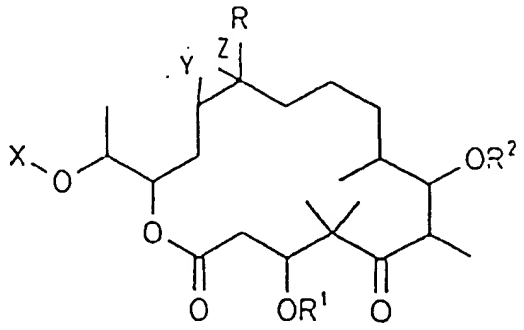
4

wherein

R = H, C_{1-4}alkyl; R^1, R^2, R^3, R^4, R^5 = H, C_{1-6}alkyl, C_{1-6}acyl, benzoyl, C_{1-4}trialkylsilyl, benzyl, phenyl, or benzyl or phenyl each substituted by C_{1-6}alkoxy, C_6alkyl, hydroxy or by halogen; the alkyl and acyl groups contained in the radicals are straight-chain or

branched radicals, X represents oxygen, NOR^3, $N\text{-}NR^4R^5$ or $N\text{-}NHCONR^4R^5$, wherein the radicals R^3 to R^5 are as defined above and R^4 and R^5 may also together form an alkylene group having from 2 to 6 carbon atoms; and
Y and Z are as defined according to claim 1.

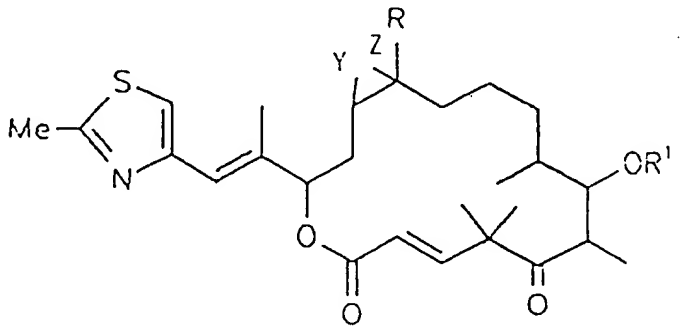
5. Epothilon derivative of formula 5



5

wherein
R = H, $C_{1\text{-}4}$alkyl; R^1, R^2 = H, $C_{1\text{-}6}$alkyl, $C_{1\text{-}6}$acyl, benzoyl, $C_{1\text{-}4}$trialkylsilyl, benzyl, phenyl, or benzyl or phenyl each substituted by $C_{1\text{-}6}$alkoxy, C_6alkyl, hydroxy or by halogen; the alkyl and acyl groups contained in the radicals are straight-chain or branched radicals, and X represents hydrogen, $C_{1\text{-}18}$alkyl, $C_{1\text{-}18}$acyl, benzyl, benzoyl or cinnamoyl, and
Y and Z are as defined according to claim 1.

6. Epothilon derivative of formula 6

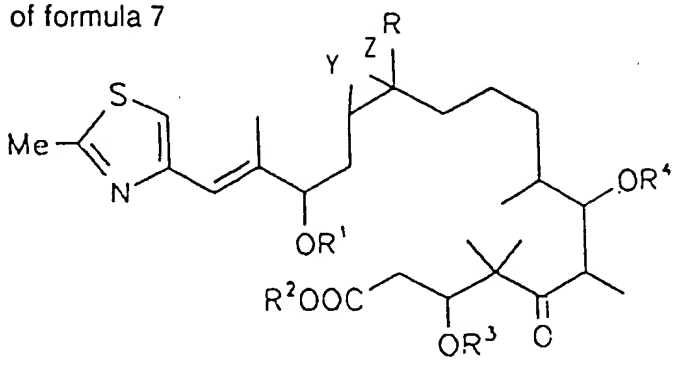


6

wherein
R = H, $C_{1\text{-}4}$alkyl and R^1 = H, $C_{1\text{-}6}$alkyl, $C_{1\text{-}6}$acyl, benzoyl, $C_{1\text{-}4}$trialkylsilyl, benzyl, phenyl, or benzyl or phenyl each substituted by $C_{1\text{-}6}$alkoxy, C_6alkyl, hydroxy or by halogen; the

alkyl and acyl groups contained in the radicals are straight-chain or branched radicals; and Y and Z are as defined according to claim 1.

7. Epothilon derivative of formula 7



7

wherein

R = H, C_{1-4}alkyl and R^1, R^2, R^3, R^4 = H, C_{1-6}alkyl, C_{1-6}acyl, benzoyl, C_{1-4}trialkylsilyl, benzyl, phenyl, or benzyl or phenyl each substituted by C_{1-6}alkoxy, C_{6}alkyl, hydroxy or by halogen; the alkyl and acyl groups contained in the radicals are straight-chain or branched radicals; and Y and Z are as defined according to claim 1.

8. Process for the preparation of an epothilon derivative of formula 7 according to claim 7, characterised in that epothilon A, epothilon B, a 3-OH-protected derivative thereof or a 7-OH-protected derivative thereof is

(a) enzymatically hydrolysed, especially with an esterase or lipase, or

(b) hydrolysed in an alkaline medium, especially with sodium hydroxide in a methanol/water mixture,

and the epothilon derivative of formula 7 is obtained and isolated.

9. Process for the preparation of an epothilon derivative of formula 2 according to claim 2, characterised in that an epothilon derivative of formula 7 according to claim 7 or in the form of the product of the process according to claim 8 is converted

(a) according to the Yamaguchi method, or

(b) according to the Corey method, or

(c) according to the Kellogg method

to form the epothilon derivative of formula 2 and that conversion product is isolated.

10. Process for the preparation of epothilon A and/or 12,13-bisepi-epothilon A, characterised in that epothilon C is epoxidised, especially with dimethyldioxirane or with a peracid.

11. Process for the preparation of epothilon B and/or 12,13-bisepi-epothilon B, characterised in that epothilon D is epoxidised, especially with dimethyldioxirane or with a peracid.

12. Composition for plant protection in agriculture and forestry and/or in horticulture, consisting of one or more of the compounds according to any one of the preceding claims or of one or more of those compounds together with one or more customary carrier(s) and/or diluent(s).

13. Therapeutic composition, especially for use as a cytostatic agent, consisting of one or more of the compounds according to one or more of claims 1 to 7 or of one or more of the compounds according to one or more of claims 1 to 7 together with one or more customary carrier(s) and/or diluent(s).

Abstract

The present invention relates to epothilon derivatives and to their use.